SUMMARY OF THE INVENTION

[0018] In one aspect, the invention provides a method of obtaining a nucleic acid segment providing a desired level of suppression of a target gene, comprising: a) obtaining a starting nucleic acid molecule substantially complementary to a target gene; b) preparing a plurality of nucleic acid segments from the starting nucleic acid molecule; c) assaying the nucleic acid segments for the ability to suppress expression of the target gene when expressed as a dsRNA in a cell comprising the target gene; and d) identifying at least a first nucleic acid segment from the plurality of nucleic acid segments that provides a desired level of suppression of the target gene when expressed as a dsRNA. In the method, the nucleic acid segments may comprise from about 21 to about 26 contiguous nucleotide portions of said starting nucleic acid molecule, including about 22, 23, 24, and 25 nucleotide portions. In certain embodiments, the segments comprise overlapping portions of said starting nucleic acid molecule and in specific embodiments may be adjoining segments. In further embodiments, the nucleic acid segments may be defined as comprising from about 0.1% to about 98% of said target gene, for example, including about 0.2%, 0.4%, 0.75%, 2%, 5%, 10%, 15%, 25%, 40%, 60%, 75% and 90%.

[0019] In one embodiment of the invention, nucleic acid segments may be ranked according to the level of suppression of the target gene obtained when the nucleic acid segments are expressed as dsRNA. The desired level of suppression of the target gene may be from about 1% to about 100% suppression of the expression of said target gene. In certain embodiments, the desired level of suppression may be complete suppression or incomplete suppression of the target gene. In specific embodiments, the target gene may be a plant, insect, fungal, bacterial or vertebrate organism, including a crop pest or pathogen gene. Assaying the nucleic acid segments for the ability to suppress the target gene may comprise expressing the segments as a dsRNA in a cell comprising the target gene and determining the level of suppression of the target gene. In one embodiment, this may comprise calculating a Reynolds score for the nucleic acid segments. In another embodiment, assaying the nucleic acid segments for the ability to suppress the target gene comprises providing said segments as dsRNA molecules in the diet of an organism comprising the target gene and determining the level of suppression of the target gene. Determining the level of suppression of the target gene may comprise observing morbidity, mortality, or stunting of said organism.

[0020] In another aspect, the invention provides a method of suppressing the expression of a target gene in a cell comprising a) obtaining a nucleic acid segment according to a method provided herein; and b) providing a dsRNA expressed from the nucleic acid to a host cell comprising the target gene to suppress the expression of the target gene. In the method, providing the dsRNA expressed from the nucleic acid segment to the host cell may comprise expressing the nucleic acid segment in the host cell in sense and antisense orientation. Providing the dsRNA expressed from the nucleic acid segment to the host cell may comprise providing a diet comprising the dsRNA to the cell or an organism comprising the cell and allowing the cell to take up the dsRNA. In one embodiment, the host cell is a pest cell and providing the dsRNA expressed from the nucleic acid to the pest cell comprises expressing the dsRNA in a plant cell and allowing a pest comprising the cell to feed on the plant cell. In specific embodiments, suppressing the expression of the target gene in the pest cell is manifested by a phenotypic effect on said cell or the pest comprising the cell. The phenotypic effect may be programmed cell death.

[0021] In yet another aspect, the invention provides a method for modulating the expression of at least a first gene in an organism comprising (a) providing as a dsRNA at least a first nucleic acid segment obtained by a method of the invention to said organism, wherein said dsRNA segment is specific for said gene in said organism; and (b) observing a phenotypic effect in said organism. In the method, the phenotypic effect may be selected from the group consisting of cessation of vegetative growth, cessation of reproductive growth, cessation of feeding, mortality, morbidity, stunting, paralysis, inhibition of sexual reproduction, molt inhibition, flightless, and failure to emerge from pupal stage.

[0022] In yet another aspect, the invention provides a method for modulating the level of expression of a gene in a plant pest comprising providing in the diet of said pest at least a first dsRNA molecule, and observing a phenotypic effect of suppression of one or more genes in said pest, wherein said dsRNA molecule is produced from a nucleotide sequence that exhibits substantial homology with a corresponding DNA sequence of one or more essential genes in said pest, and wherein said nucleotide sequence is a nucleic acid segment identified according to a method provided herein.

[0023] In still yet another aspect, the invention provides a method for inhibiting plant pest infestation comprising expressing a dsRNA molecules obtained according to a method of the invention in a transgenic plant and providing the plant or a part or tissue thereof to one or more pests comprising said nucleotide sequence, and observing a phenotypic effect in said organism, wherein the phenotypic effect is sufficient to inhibit infestation of said transgenic plant by said pest.

[0024] In still yet another aspect, the invention provides a method for protecting a plant from pest infestation comprising expressing a dsRNA molecules obtained according to the invention in a transgenic plant, providing said plant or a part or tissue thereof to one or more pests comprising said nucleotide sequence, and observing a phenotypic effect in the organism, wherein the phenotypic effect is sufficient to inhibit infestation of the transgenic plant by the pest. The invention also provides a plant protected from pest infestation according to any of the methods described herein, as well as a plant regenerated from such a cell, and also a seed or progeny produced from such a plant, wherein said seed or progeny comprises a nucleotide sequence obtained according to the invention.

[0025] In still yet another aspect, the invention provides a method of producing an expression construct for expressing a dsRNA with reduced transgene silencing in a plant cell, comprising: (a) preparing an expression construct comprising a first sequence, a second sequence, and a third polynucleotide sequence, wherein the third polynucleotide sequence is linked to the first polynucleotide sequence by the second polynucleotide sequence and the third polynucleotide sequence is substantially the reverse complement of the first polynucleotide sequence; and (b) introducing an intron into at least one of the first and third polynucleotide sequences or introducing said expression construct into the intron, wherein the first and third polynucleotide sequences hybridize when transcribed into RNA and form a dsRNA molecule stabilized by the second polynucleotide sequence after intron splicing, and wherein the expression construct exhibits reduced trans-